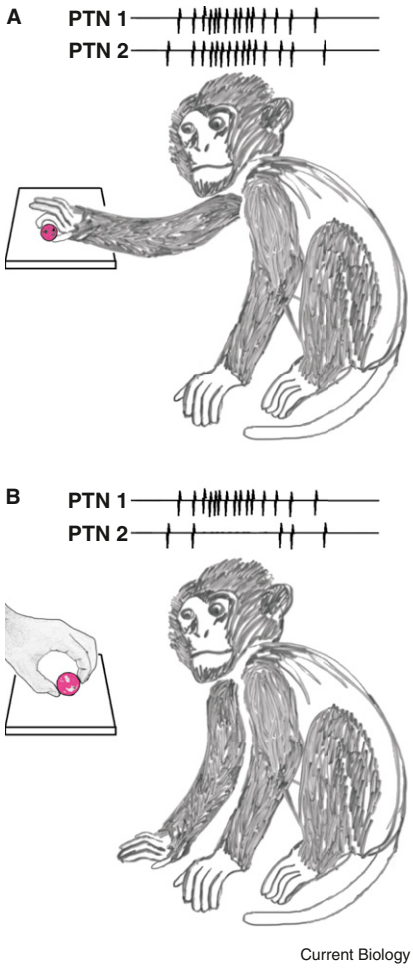

Figure 1. Mirror pyramidal tract neurons.

(A) Action execution. Two neurons in the motor cortex that send their axons to the spinal cord via the pyramidal tract (pyramidal tract neurons, PTNs) each fire a burst of action potentials (PTN 1 and PTN 2) as a monkey executes a precision pinch, grasping a raisin between the tips of the thumb and index finger. (B) Action observation. Each pyramidal tract neuron also responds as the monkey watches a human grasp a raisin with a precision pinch. But while PTN 1 fires another burst, the firing of PTN 2 is comparatively suppressed. The total excitation delivered to the spinal cord by the pyramidal tract neurons therefore is less during action observation, when the monkey does not execute the movement. (Monkey drawn by A. Goodman.)

system to execute the upcoming movement as quickly and efficiently as possible when the "Go" cue arrives.

Our understanding of mirror neurons may evolve in a similar direction as future studies explore additional possibilities. During action observation, visual inputs lead to activation of mirror neurons but not to movement execution. Perhaps mirror neurons can be activated as well by internal inputs. Humans can imagine making a movement — 'motor imagery' — without actually performing the movement. Studies using functional magnetic resonance imaging show activation in the same part of M1 during motor imagery as occurs during actual execution of the same movement [14]. Perhaps just as visual inputs activate mirror neurons during action observation, internal inputs activate mirror neurons during motor imagery.

Furthermore, by imagining the same movement being performed over and over — 'mental rehearsal' — humans may actually improve their performance in tasks ranging from the seemingly non-cognitive, such as weightlifting, to the highly skilled, such as surgery and piano playing. Improved execution indicates that the mental rehearsal induced some degree of motor learning, presumably reflecting changes in synaptic strengths resulting from activity-dependent plasticity. If mirror pyramidal tract neurons eventually are found to be activated during mental imagery and mental rehearsal, then some of this synaptic plasticity may be occurring in the spinal cord [15]. Finally, both humans and monkeys can learn to perform a complex movement by watching others perform the movement. Mirror pyramidal tract neurons may cause activity-dependent synaptic plasticity in the spinal cord that helps Mr Bananas learn to perform a new movement after watching Fred: monkey see, monkey do.

References

1. Vigneswaran, G., Philipp, R., Lemon, R.N., and Kraskov, A. (2013). M1 corticospinal mirror neurons and their role in movement suppression during action observation. Curr. Biol. *23*, 236–243.
2. di Pellegrino, G., Fadiga, L., Fogassi, L., Gallese, V., and Rizzolatti, G. (1992). Understanding motor events: a neurophysiological study. Exp. Brain Res. *91*, 176–180.
3. Gallese, V., Fadiga, L., Fogassi, L., and Rizzolatti, G. (1996). Action recognition in the premotor cortex. Brain *119*, 593–609.
4. Casile, A. (2012). Mirror neurons (and beyond) in the macaque brain: An overview of twenty years of research. Neurosci. Lett. http://dx.doi.org/10.1016/j.neulet.2012.11.003, Epub ahead of print.
5. Rizzolatti, G., Fadiga, L., Gallese, V., and Fogassi, L. (1996). Premotor cortex and the recognition of motor actions. Cogn. Brain Res. *3*, 131–141.
6. Umilta, M.A., Kohler, E., Gallese, V., Fogassi, L., Fadiga, L., Keysers, C., and Rizzolatti, G. (2001). I know what you are doing: A neurophysiological study. Neuron *31*, 155–165.
7. Kohler, E., Keysers, C., Umilta, M.A., Fogassi, L., Gallese, V., and Rizzolatti, G. (2002). Hearing sounds, understanding actions: action representation in mirror neurons. Science *297*, 846–848.
8. Ferrari, P.F., Gallese, V., Rizzolatti, G., and Fogassi, L. (2003). Mirror neurons responding to the observation of ingestive and communicative mouth actions in the monkey ventral premotor cortex. Eur. J. Neurosci. *17*, 1703–1714.
9. Kraskov, A., Dancause, N., Quallo, M.M., Shepherd, S., and Lemon, R.N. (2009). Corticospinal neurons in macaque ventral premotor cortex with mirror properties: a potential mechanism for action suppression? Neuron *64*, 922–930.
10. Tanji, J., and Evarts, E.V. (1976). Anticipatory activity of motor cortex neurons in relation to direction of an intended movement. J. Neurophysiol. *39*, 1062–1068.
11. Weinrich, M., Wise, S.P., and Mauritz, K.H. (1984). A neurophysiological study of the premotor cortex in the rhesus monkey. Brain *107*, 385–414.
12. Buford, J.A., and Davidson, A.G. (2004). Movement-related and preparatory activity in the reticulospinal system of the monkey. Exp. Brain Res. *159*, 284–300.
13. Prut, Y., and Fetz, E.E. (1999). Primate spinal interneurons show pre-movement instructed delay activity. Nature *401*, 590–594.
14. Munzert, J., Lorey, B., and Zentgraf, K. (2009). Cognitive motor processes: the role of motor imagery in the study of motor representations. Brain Res. Rev *60*, 306–326.
15. Wolpaw, J.R. (2010). What can the spinal cord teach us about learning and memory? Neuroscientist *16*, 532–549.

Department of Neurology, University of Rochester, 601 Elmwood Ave, Box 673, Rochester, NY 14642, USA.
E-mail: mhs@cvs.rochester.edu

http://dx.doi.org/10.1016/j.cub.2013.01.004

Plant Development: Brassinosteroids Go Out of Bounds

Patterning in plants requires defining boundary domains that separate and organize the development of the neighboring organs. Two papers now show how the interplay between brassinosteroid phytohormones and frontier genes contributes to boundary formation in plants.

Nicolas Arnaud and Patrick Laufs

The formation of boundaries is a recurrent process during both animal and plant development [1,2]. Boundaries act as a frontier between two different cell types, thus allowing cell specialization and the apparition of

new functions. In addition, boundaries also often act as organizing centers, providing information to the neighboring cells to control their fate. Therefore, the setup of boundaries is a crucial step in the development of multicellular organisms and enables the generation of tissues and organs with specific functions and shapes. Two recent papers published in *Proceedings of the National Academy of Sciences* describe a new role for brassinosteroid (BR) phytohormones in the formation of boundaries in plants [3,4].

One of the best characterized boundary domains in plants is the boundary that separates the primordium of lateral organs, such as leaves, from the pool of undifferentiated cells, the meristem, from which it originates [2,5]. This boundary forms a groove between the meristem and organ primordia or between neighboring organ primodia and is formed by a stretch of cells with particular shapes and reduced growth. Transcription factors encoded by genes such as *CUP-SHAPED COTYLEDON* (*CUC1*, *CUC2* and *CUC3*), *LATERAL ORGAN FUSION* (*LOF1*), *LATERAL ORGAN BOUNDARIES* (*LOB*) or *JAGGED LATERAL ORGAN* (*JLO*) specify the boundary domain [2,5]. Inactivation of some of these genes, such as the *CUC* genes, leads to organ fusion, consistent with their role in the repression of cell proliferation to allow organ separation [6]. Mutations of these genes also lead to defects outside the boundary domain as exemplified by their effect on the expression of the type I *KNOX* genes. Type I *KNOX* genes are expressed in the meristem where they prevent cell differentiation and are repressed in the founder cells of lateral organs [7]. Mutation of the *JLO* boundary gene leads to ectopic type I *KNOX* expression in the developing lateral organs [8], whereas reduced type I *KNOX* expression is observed in *cuc* mutants leading to defective meristems [9]. Therefore, the boundary domain acts both locally to allow organ separation and at a distance to control meristem and organ primordium development. How the boundary domain is set up both at the molecular and cellular levels is far from being understood, though some mechanisms controlling the expression pattern of the boundary genes have been identified and some of their targets characterized [2,10].

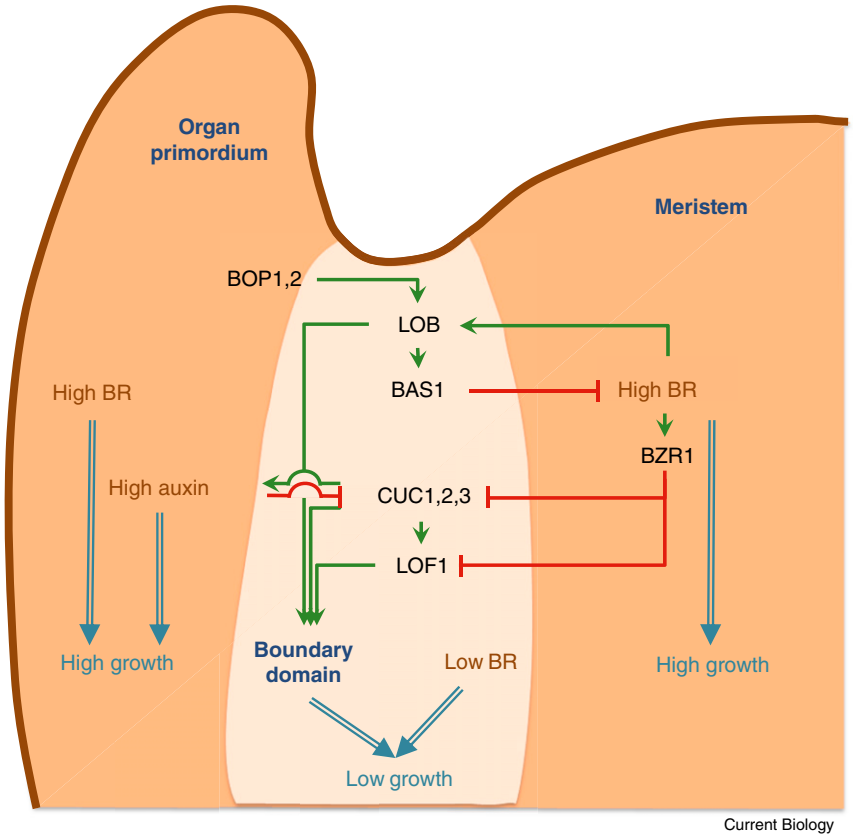

Figure 1. BR and the boundary domain in plants.

BRs and primordium expression of *BOP1* and *BOP2* activate *LOB* expression in the boundary domain separating the organ primordium from the pool of undifferentiated cells, the meristem. *LOB* reduces BR signaling, in particular via the activation of the BR-inactivating enzyme *BAS1*. In turn, low BR leads to a derepression of the *CUC* and *LOF* genes and low growth, thus generating the boundary domain. Cross-talk between BR and auxin, and between auxin and boundary genes, in particular the *CUC* genes, contributes to the patterning of the boundary domain.

BRs represent a major class of plant steroid hormones promoting growth in a variety of developmental processes [11]. BRs bind to the plasma membrane receptor kinase BRI1 and act via the BIN2 kinase and the BSU1 phosphatase to modulate the DNA-binding activities of BZR1 and BZR2/BES1 transcription factors on their target genes [12,13]. Mutants insensitive to BRs are dwarf [11], and at the cellular level BRs promote growth by modulating both cell expansion and cell division [14]. Many targets of BZR1 are involved in cell wall and cytoskeleton reorganization as well as water and ion fluxes, which are important processes controlling cell expansion [15]. In addition, *bri1* mutants have reduced root meristem size due to decreased mitotic activity, which can be rescued by over expression of *CycD3;1* [16].

In a three-step demonstration, Gendron *et al.* [3] and Bell *et al.* [4] show that BRs contribute to define the boundary domain, at both the molecular and cellular levels. The first step was the demonstration that BRs antagonize boundary formation between organs: exogenous BR application, overexpression of the BR biosynthetic gene *DWF4*, or expression of a dominant BR-hypersensitive form of the BZR1 transcription factor lead to organ separation defects. These defects include fusion between cotyledons or floral organs and between an axillary branch and its subtending cauline leaf. Since the nuclear accumulation of the BZR1 protein is regulated by BR-dependent phosphorylation, Gendron *et al.* used BZR1 distribution

in the meristem as a proxy for BR activity, thus showing that BR signaling is reduced in the boundary domain. How could this reduced BR signaling contribute to meristem patterning? As BRs are positive regulators of cell growth/division, lower BR signaling in the boundary domain could contribute to the reduced growth of this domain. Gendron *et al.* revealed an additional mechanism: in the second step of their demonstration, they showed that BRs repress the expression of the *CUC1*, *CUC2*, *CUC3* and *LOF1* boundary genes, leading to the hypothesis that the specific expression of these genes may result from their local derepression due to lower BR signaling. Indeed, Bell *et al.* showed in the third step of the demonstration how a domain with lower BR signaling could be generated. The key here was the identification of the targets of the LOB transcription factor. Among the genes whose expression was modified following ectopic LOB expression, 60% were previously shown to be modulated by BRs, providing a molecular basis for the observation that LOB modulates BR responses. Among these genes, *BAS1,* which encodes a BR-inactivating enzyme, was shown to be directly activated by LOB. *BAS1* and *LOB* expression overlap in the boundary region that separates an axillary branch from its subtending cauline leaf, and *LOB* inactivation leads to fusion of these two structures. Very elegantly, Bell and collaborators showed that expressing BAS1 under the control of the *LOB* promoter was sufficient to suppress the organ fusion defect of the *lob* mutant, showing that reducing BR locally is sufficient for the formation of a functional boundary domain. Therefore, the following model can be proposed (Figure 1): expression of the *LOB* gene leads to the repression of BR signaling, partly through the activation of the BAS1 BR-inactivating enzyme. Reduced BR signaling in turn limits cell growth of the boundary domain for proper organ separation while promoting the expression of the other boundary genes such as *CUC* and *LOF* genes.

While *LOB* is expressed in the boundary where BR signaling is low, Bell *et al.* also showed that BRs activate LOB. To explain this apparent contradiction, one could envisage that a local burst of BR signaling leads to LOB activation, which in turn inactivates BR signaling. While there is for the moment no evidence for such a scenario, other mechanisms may contribute to initiate *LOB* expression. *LOB* has been shown to be activated by *BOP1/2,* which are expressed in a layer of cells adjacent to the lateral organ boundary [17]. Hormones other than BRs may also be at play in the formation of boundary domains. Indeed, *JLO* and *AS2*, two members of the LBD family to which *LOB* also belongs [18], coordinate transport of the phytohormone auxin *via* the control of the expression of the auxin efflux ransporter PIN [8]. In leaves, *CUC2* promotes the generation of *PIN*-dependent auxin maxima, while auxin represses *CUC2* expression in a regulatory loop [19]. Taken together, these results reveal a close interplay between auxin and BR in the formation of the boundary domain. The next challenge will be to further analyze this interplay and identify the molecular mechanisms at play. This could be inspired, for instance, by previous work demonstrating a synergistic effect between auxin and BRs during photomorphogenesis in which it was shown that the phosphorylation of the auxin response factor ARF2 by the BR response repressor BIN2 results in the loss of DNA-binding capacities of ARF2 [20].

References

1. Dahmann, C., Oates, A.C., and Brand, M. (2011). Boundary formation and maintenance in tissue development. Nat. Rev. Genet. *12*, 43–55.
2. Rast, M.I., and Simon, R. (2008). The meristem-to-organ boundary: more than an extremity of anything. Curr. Opin. Genet. Dev. *18*, 287–294.
3. Bell, E.M., Lin, W.C., Husbands, A.Y., Yu, L., Jaganatha, V., Jablonska, B., Mangeon, A., Neff, M.M., Girke, T., and Springer, P.S. (2012). Arabidopsis LATERAL ORGAN BOUNDARIES negatively regulates brassinosteroid accumulation to limit growth in organ boundaries. Proc. Natl. Acad. Sci. USA *109*, 21146–21151.
4. Gendron, J.M., Liu, J.S., Fan, M., Bai, M.Y., Wenkel, S., Springer, P.S., Barton, M.K., and Wang, Z.Y. (2012). Brassinosteroids regulate organ boundary formation in the shoot apical meristem of Arabidopsis. Proc. Natl. Acad. Sci. USA *109*, 21152–21157.
5. Aida, M., and Tasaka, M. (2006). Morphogenesis and patterning at the organ boundaries in the higher plant shoot apex. Plant Mol. Biol. *60*, 915–928.
6. Aida, M., Ishida, T., Fukaki, H., Fujisawa, H., and Tasaka, M. (1997). Genes involved in organ separation in Arabidopsis: an analysis of the cup-shaped cotyledon mutant. Plant Cell *9*, 841–857.
7. Hay, A., and Tsiantis, M. (2010). KNOX genes: versatile regulators of plant development and diversity. Development *137*, 3153–3165.
8. Rast, M.I., and Simon, R. (2012). Arabidopsis JAGGED LATERAL ORGANS acts with ASYMMETRIC LEAVES2 to coordinate KNOX and PIN expression in shoot and root meristems. Plant Cell *24*, 2917–2933.
9. Aida, M., Ishida, T., and Tasaka, M. (1999). Shoot apical meristem and cotyledon formation during Arabidopsis embryogenesis: interaction among the CUP-SHAPED COTYLEDON and SHOOT MERISTEMLESS genes. Development *126*, 1563–1570.
10. Takeda, S., Hanano, K., Kariya, A., Shimizu, S., Zhao, L., Matsui, M., Tasaka, M., and Aida, M. (2011). CUP-SHAPED COTYLEDON1 transcription factor activates the expression of LSH4 and LSH3, two members of the ALOG gene family, in shoot organ boundary cells. Plant J. *66*, 1066–1077.
11. Clouse, S.D., Langford, M., and McMorris, T.C. (1996). A brassinosteroid-insensitive mutant in Arabidopsis thaliana exhibits multiple defects in growth and development. Plant Physiol. *111*, 671–678.
12. Vert, G., and Chory, J. (2006). Downstream nuclear events in brassinosteroid signalling. Nature *441*, 96–100.
13. He, J.X., Gendron, J.M., Yang, Y., Li, J., and Wang, Z.Y. (2002). The GSK3-like kinase BIN2 phosphorylates and destabilizes BZR1, a positive regulator of the brassinosteroid signaling pathway in Arabidopsis. Proc. Natl. Acad. Sci. USA *99*, 10185–10190.
14. Mussig, C. (2005). Brassinosteroid-promoted growth. Plant Biol. *7*, 110–117.
15. Sun, Y., Fan, X.Y., Cao, D.M., Tang, W., He, K., Zhu, J.Y., He, J.X., Bai, M.Y., Zhu, S., Oh, E., *et al*. (2010). Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis. Dev. Cell *19*, 765–777.
16. Gonzalez-Garcia, M.P., Vilarrasa-Blasi, J., Zhiponova, M., Divol, F., Mora-Garcia, S., Russinova, E., and Cano-Delgado, A.I. (2011). Brassinosteroids control meristem size by promoting cell cycle progression in Arabidopsis roots. Development *138*, 849–859.
17. Ha, C.M., Kim, G.T., Kim, B.C., Jun, J.H., Soh, M.S., Ueno, Y., Machida, Y., Tsukaya, H., and Nam, H.G. (2003). The BLADE-ON-PETIOLE 1 gene controls leaf pattern formation through the modulation of meristematic activity in Arabidopsis. Development *130*, 161–172.
18. Shuai, B., Reynaga-Pena, C.G., and Springer, P.S. (2002). The lateral organ boundaries gene defines a novel, plant-specific gene family. Plant Physiol. *129*, 747–761.
19. Bilsborough, G.D., Runions, A., Barkoulas, M., Jenkins, H.W., Hasson, A., Galinha, C., Laufs, P., Hay, A., Prusinkiewicz, P., and Tsiantis, M. (2011). Model for the regulation of Arabidopsis thaliana leaf margin development. Proc. Natl. Acad. Sci. USA *108*, 3424–3429.
20. Vert, G., Walcher, C.L., Chory, J., and Nemhauser, J.L. (2008). Integration of auxin and brassinosteroid pathways by Auxin Response Factor 2. Proc. Natl. Acad. Sci. USA *105*, 9829–9834.

INRA, UMR1318, Institut Jean-Pierre Bourgin, RD10, F-78000 Versailles, France.
AgroParisTech, Institut Jean-Pierre Bourgin, RD10, F-78000 Versailles, France.
E-mail: patrick.laufs@versailles.inra.fr

http://dx.doi.org/10.1016/j.cub.2013.01.001